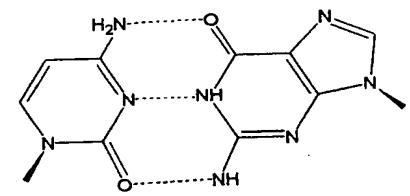
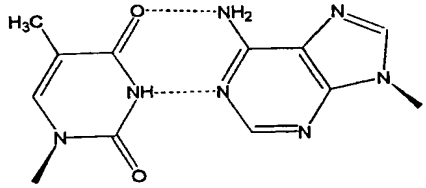
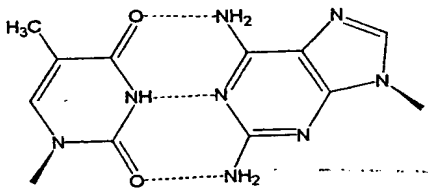
Figure 1



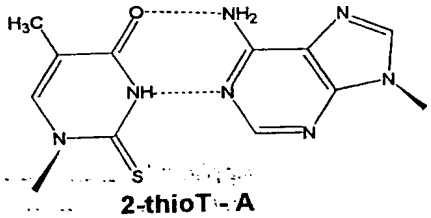
C - G



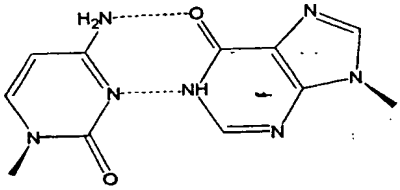
T- A



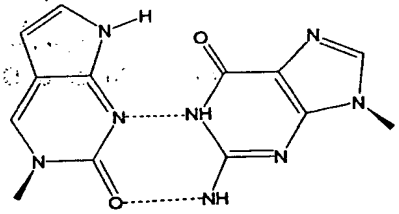
T - 2-aminoA



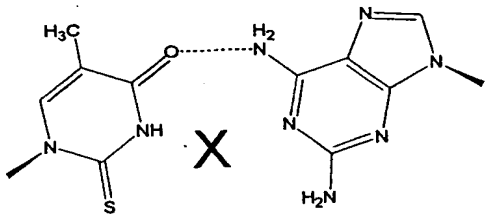
2-thioT - A



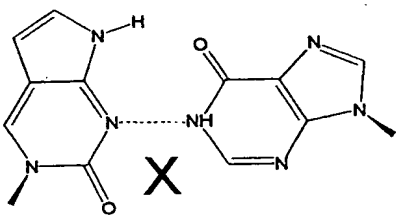
C - I



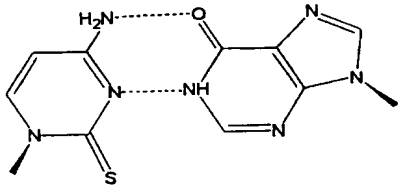
PyrroloPyr - G



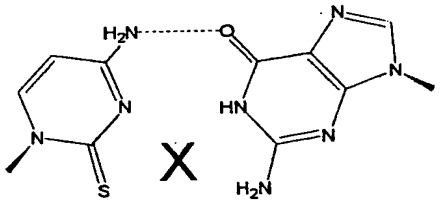
2-thioT - 2-aminoA



PyrroloPyr - I



2-thioC - I



2-thioC - G

Figure 2

A

```
5′-CGATAGGCTCTG →
3′-GCTATCCGAGACCCTGACTTGACACCTGTT-5′
```

B

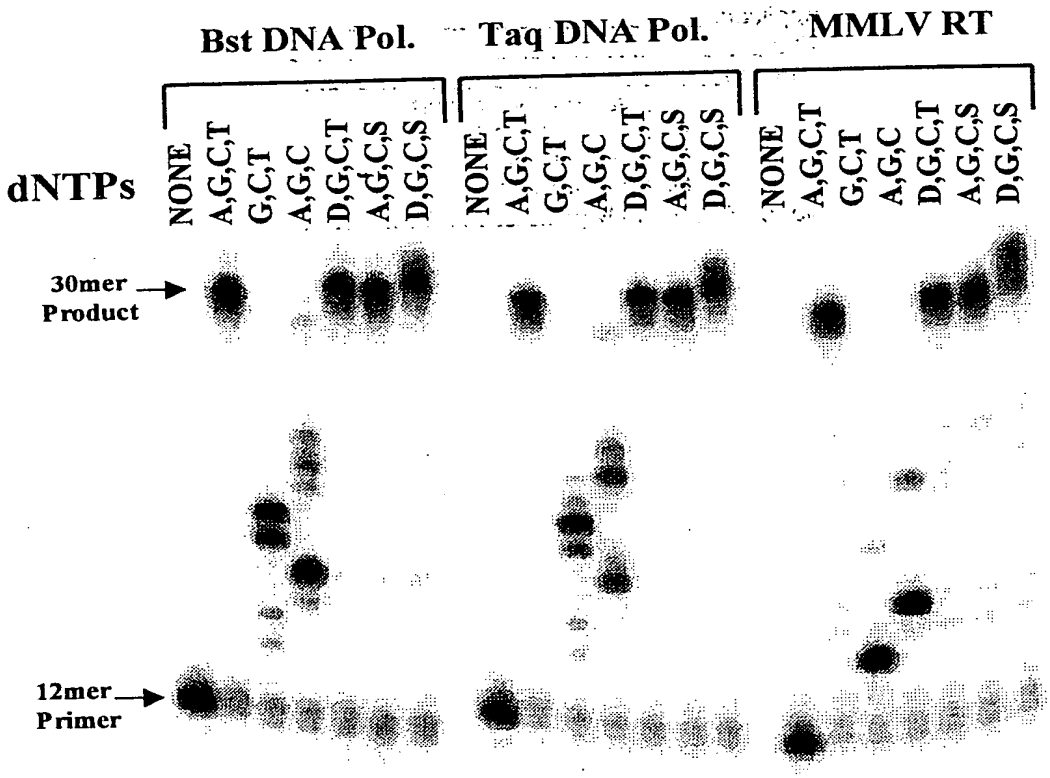
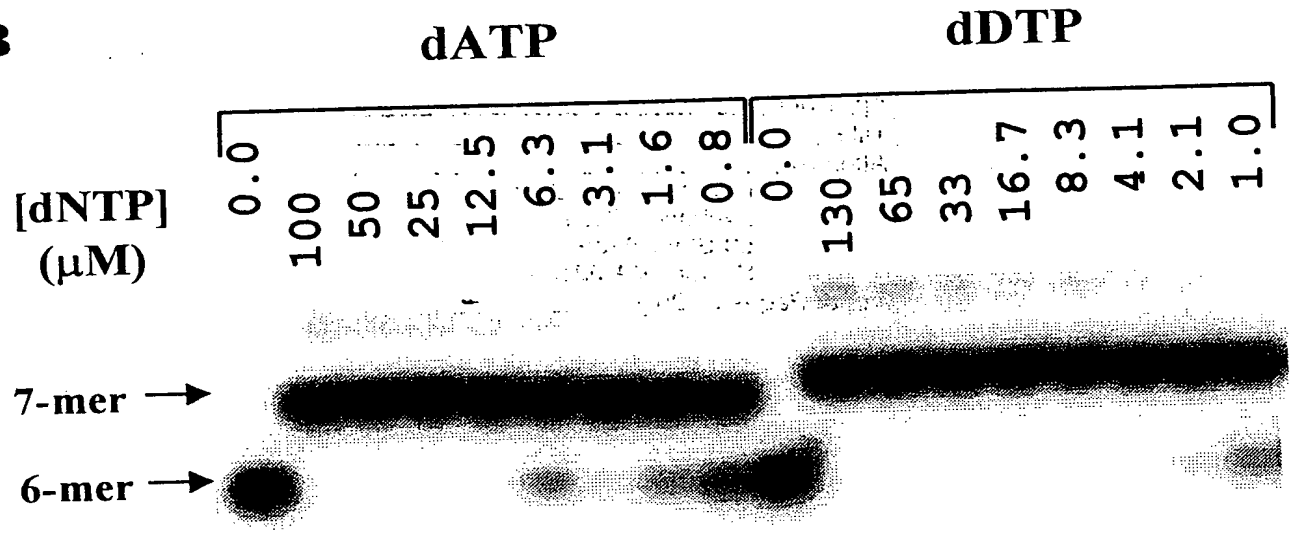


Figure 3

A

```
                    5′-GACTGA→
3′-GCTATCCGAGACACTGACTTGACACCTGTT-5′
```

B



C

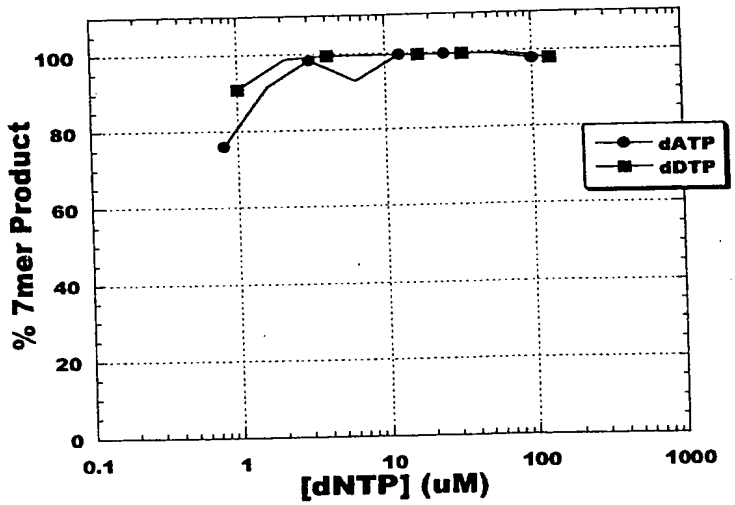
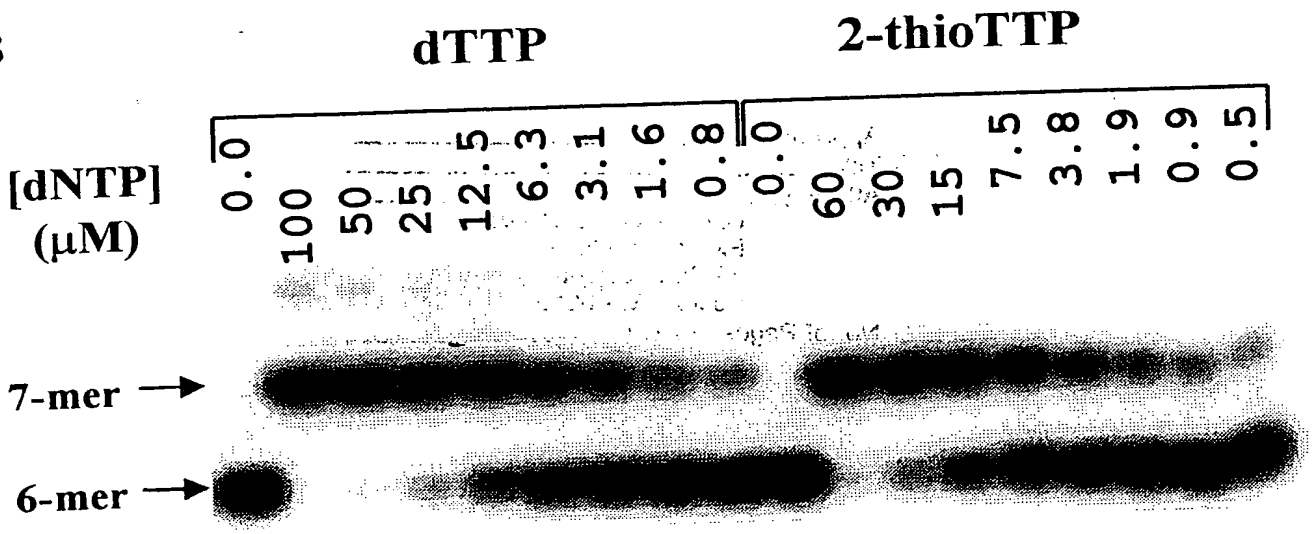


Figure 4

A


5′-GCTCTG →
3′-GCTATCCGAGACACTGACTTGACACCTGTT-5′

B


dTTP
2-thioTTP
[dNTP] (μM)
0.0
100
50
25
12.5
6.3
3.1
1.6
0.8
0.0
60
30
15
7.5
3.8
1.9
0.9
0.5
7-mer
6-mer

C

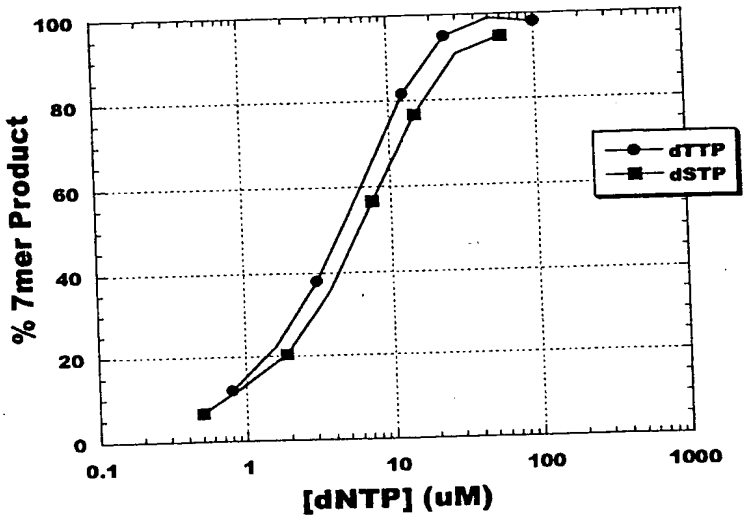
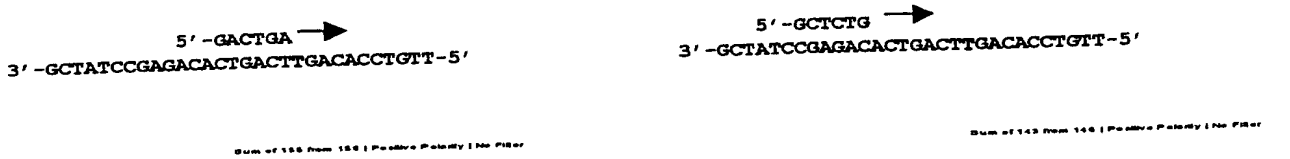
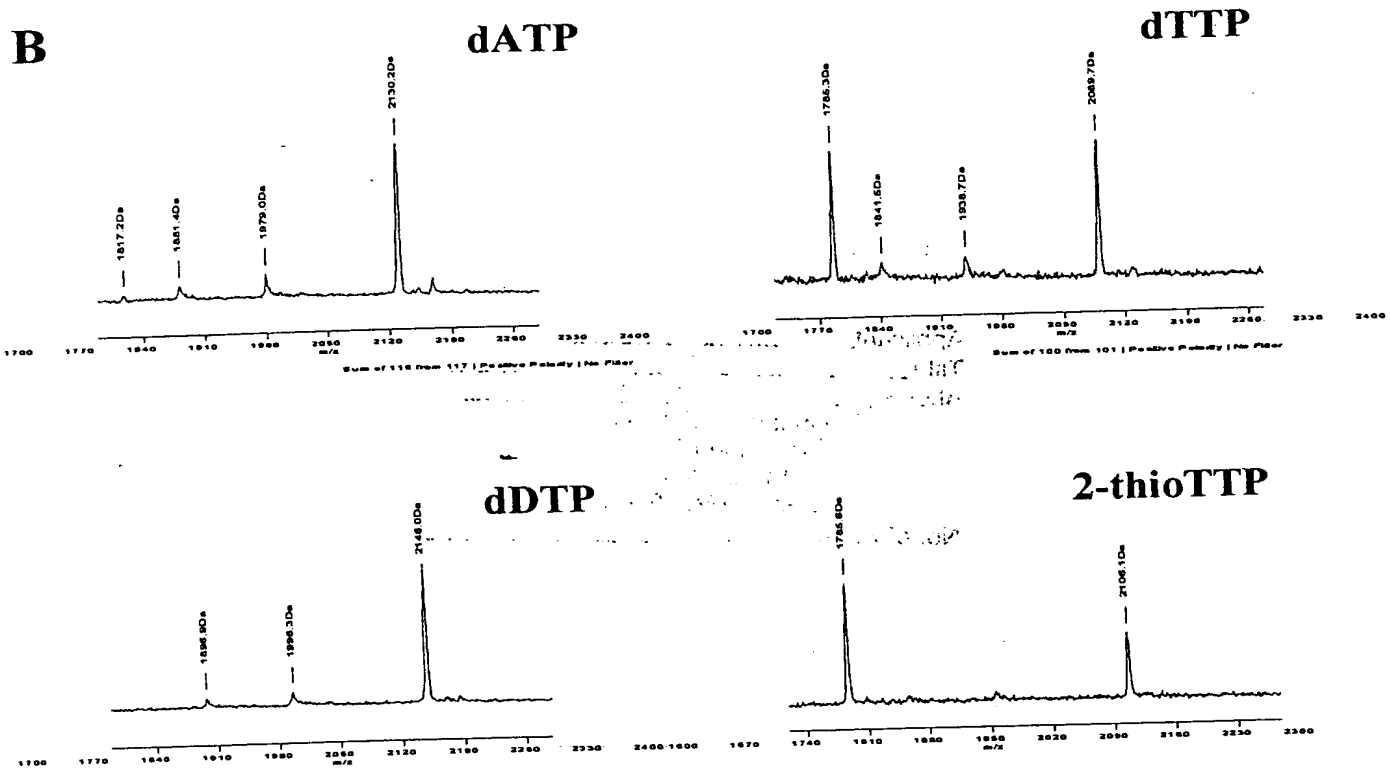

% 7mer Product
100
80
60
40
20
0
0.1
1
10
100
1000
[dNTP] (uM)
dTTP
dSTP

Figure 5

A



B



C

Extension Nucleotide	X-mer	Predicted m/z (Positive Ion)*	Measured m/z (Positive Ion)	Predicted Δ m/z	Measured Δ m/z
None	GACTGA	1816.3	nd	--	--
dATP	GACTGAA	2129.5	2130.2	+15.0	+15.8
dDTP	GACTGAD	2144.5	2146.0		
None	GCTCTG	1783.2	1785.4 ± 0.2	--	--
dTTP	GCTCTGT	2087.4	2089.7	+16.0	+16.4
d-2-thio-TTP	GCTCTGS	2103.4	2106.1		

Figure 6

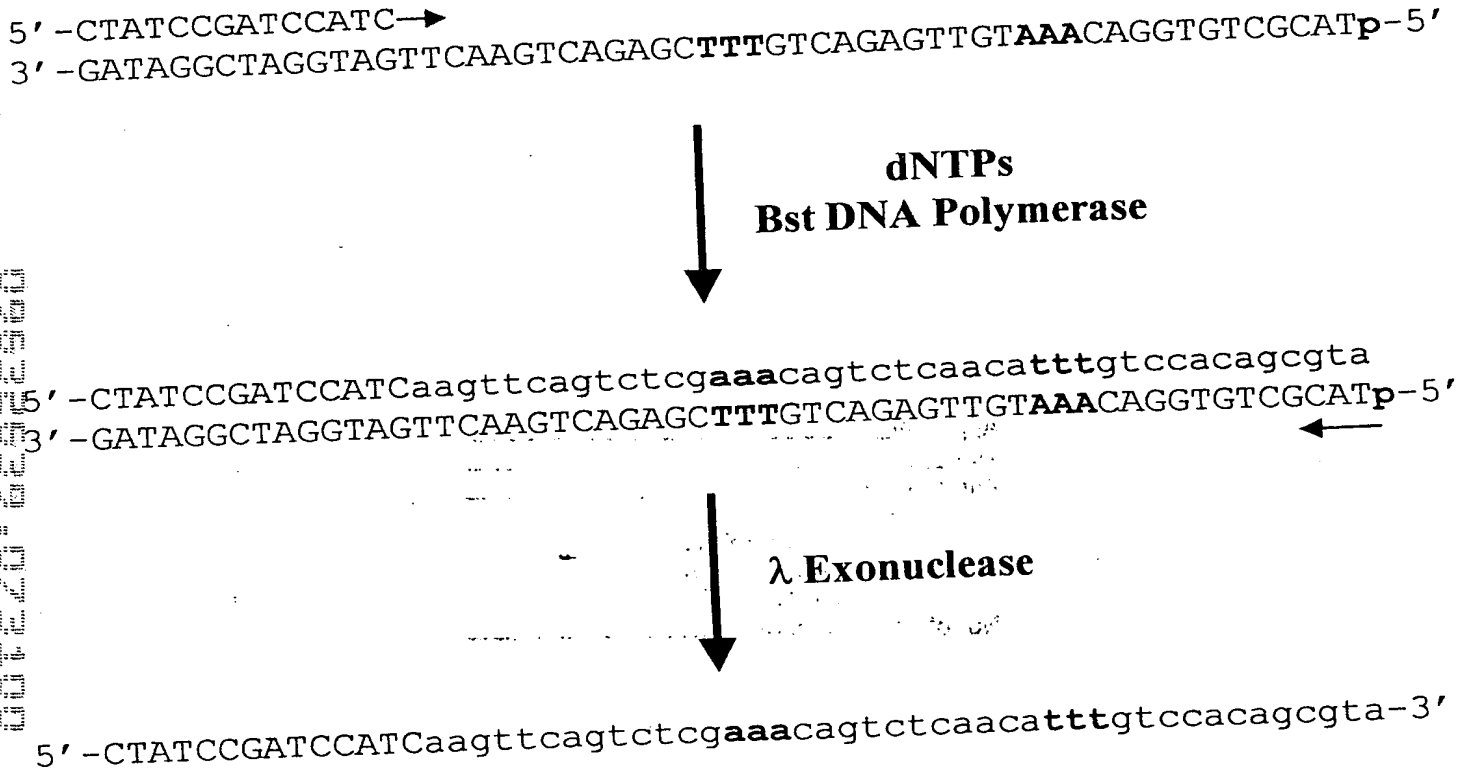

5′-CTATCCGATCCATC→
3′-GATAGGCTAGGTAGTTCAAGTCAGAGCTTTGTCAGAGTTGTAAACAGGTGTCGCATp-5′
dNTPs
Bst DNA Polymerase
5′-CTATCCGATCCATCaagttcagtctcgaaacagtctcaacatttgtccacagcgta
5′-CTATCCGATCCATCaagttcagtctcgaaacagtctcaacatttgtccacagcgta
3′-GATAGGCTAGGTAGTTCAAGTCAGAGCTTTGTCAGAGTTGTAAACAGGTGTCGCATp-5′
λ Exonuclease
5′-CTATCCGATCCATCaagttcagtctcgaaacagtctcaacatttgtccacagcgta-3′

Figure 7

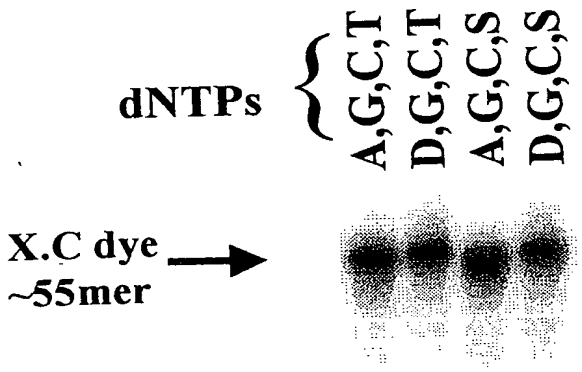


Figure 8

HP21AT

ΔG° = -12.3 kcal/mole at 37 °C
Δ H° = -82.5 kcal/mole
Δ S°= -226.3 cal/ (°K·mol)
Tm = 91.4°C

```
          10        20
5'-CTATCCGATCCATCAA          G
                  GTTCAGTCTC A
                  ||||||||||
                  CAAGTCAGAG A
3'-ATGCGACACCTGTTTA          A
        50        40        30
```

HP21DS

```
          10        20
5'-CTATCCGATCCATCDD          G
                  GSSCDGSCSC D
                  |..|.|.|.|
                  CDDGSCDGDG D
3'-DSGCGDCDCCSGSSSD          D
        50        40        30
```

HP26AT

Δ G° = -3.8 kcal/mole at 37 °C
Δ H° = -41.2 kcal/mole
Δ S°= -120.5 cal/ (°K·mol)
Tm = 68.8°C

```
          10        20
5'-CTATCCGATCCATCAA   C  T   G
                  GTT AG CTC A
                  ||| || |||
                  CAA TC GAG A
3'-ATGCGACACCTGTTTA   C  T   A
        50        40        30
```

HP26DS

```
          10        20
5'-CTATCCGATCCATCDD   C  S   G
                  GSS DG CSC D
                  |.. .| |.|
                  CDD SC GDG D
3'-DSGCGDCDCCSGSSSD   C  S   D
        50        40        30
```

HP28AT

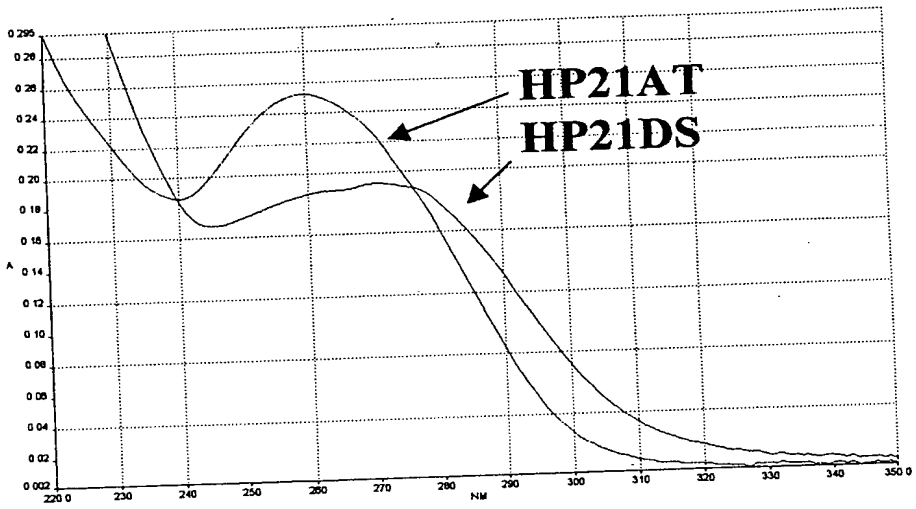
Δ G° = 0.1 kcal/mole at 37 °C
Δ H° = -27.4 kcal/mole
Δ S°= -88.6 cal/ (°K·mol)
Tm = 36.1°C

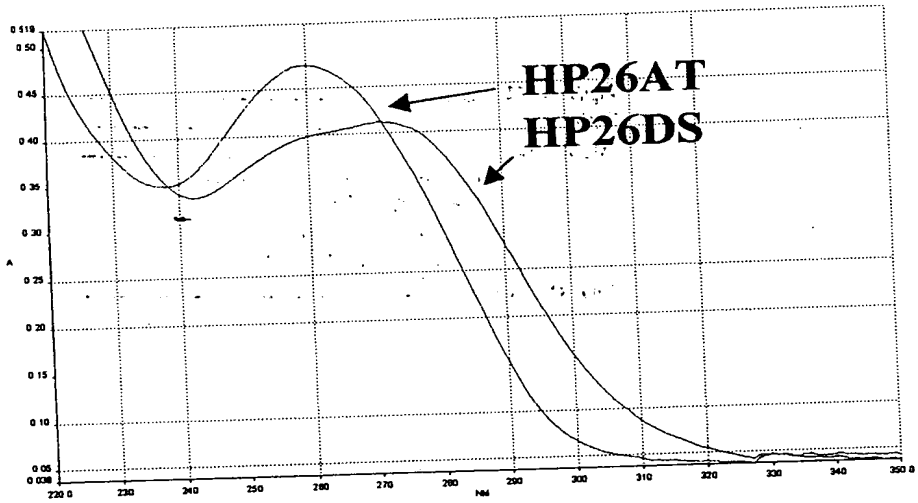
```
          10        20
5'-CTATCCGATCCATCAA   C  T  CG
                  GTT AG CT  A
                  ||| || ||
                  CAA TC GA  A
3'-ATGCGACACCTGTTTA   C  T  CA
        50        40        30
```

HP28DS

```
          10        20
5'-CTATCCGATCCATCDD   C  S  CG
                  GSS DG CS  D
                  |.. .| |.
                  CDD SC GD  D
3'-DSGCGDCDCCSGSSSD   C  S  CD
        50        40        30
```

Figure 9





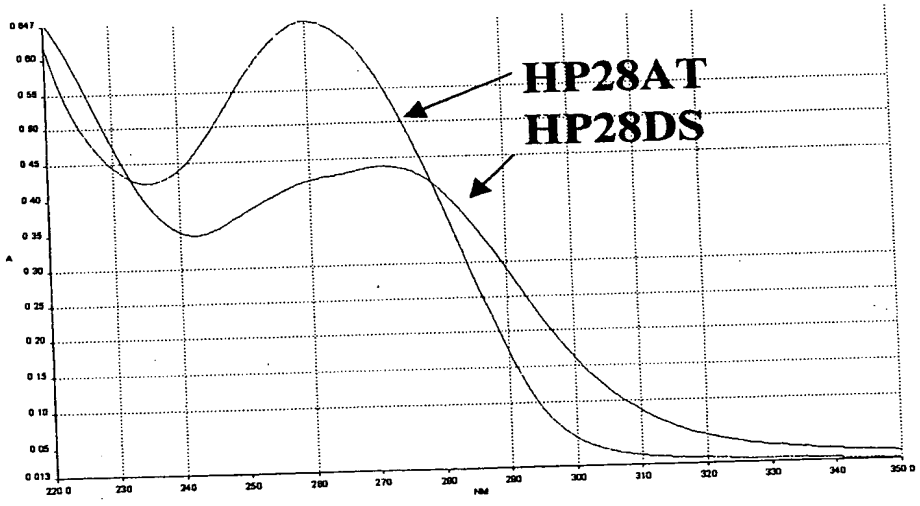
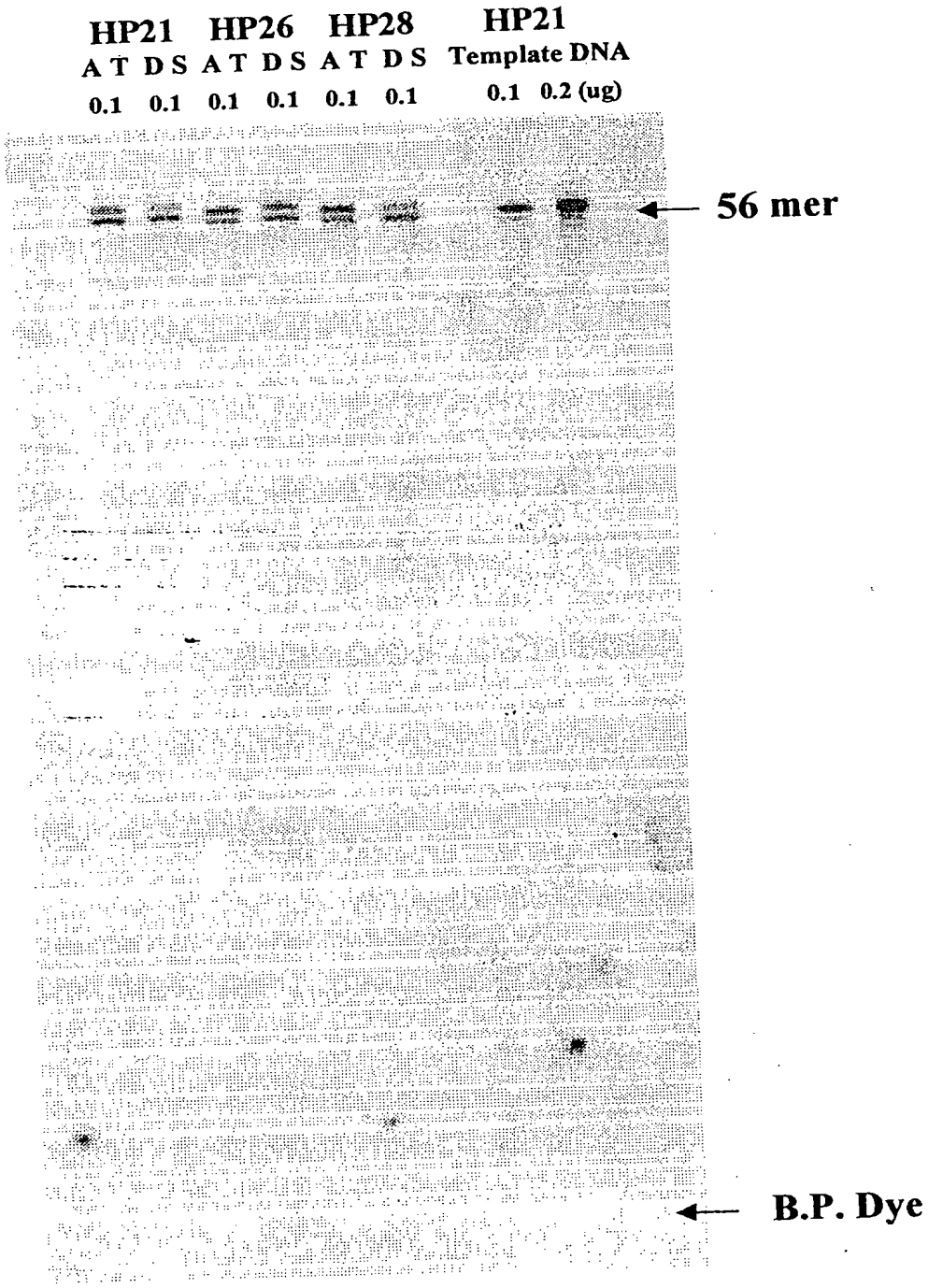


Figure 10



10% 7M Urea PAGE

Figure 11

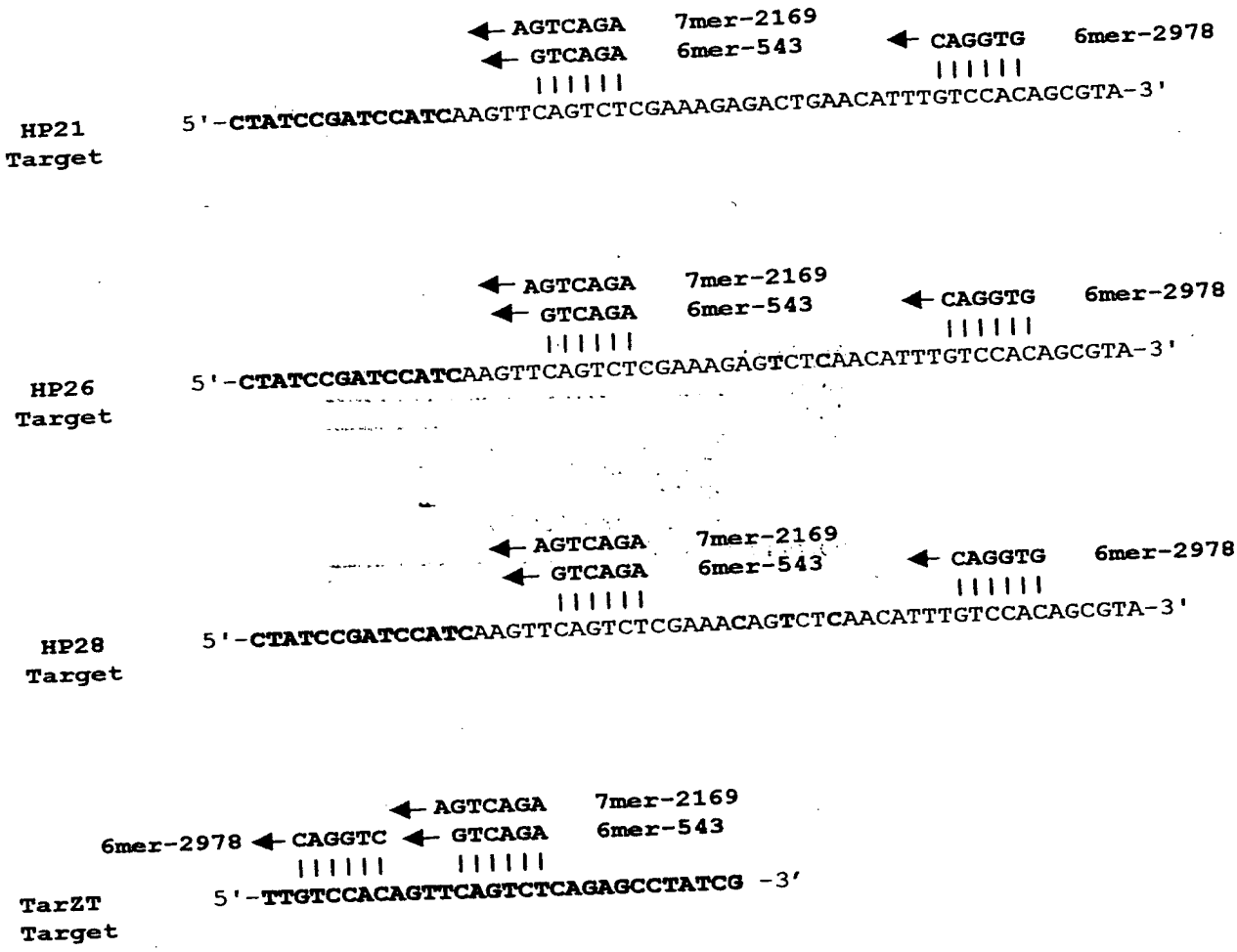
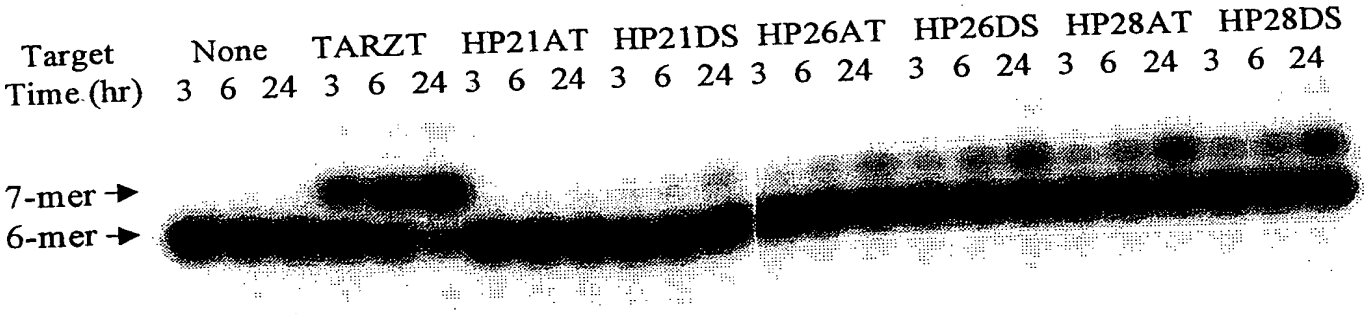
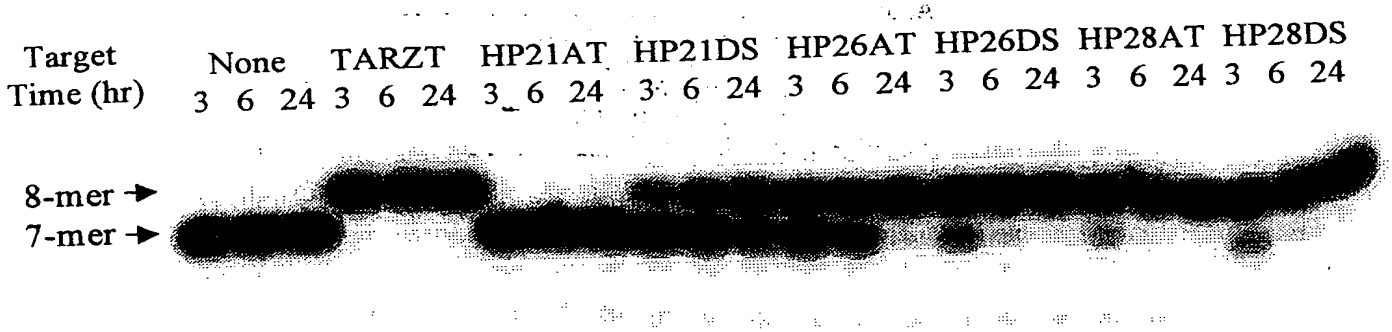

AGTCAGA
7mer-2169
GTCAGA
6mer-543
CAGGTG
6mer-2978
HP21
Target
5'-CTATCCGATCCATCAAGTTCAGTCTCGAAAGAGACTGAACATTTGTCCACAGCGTA-3'
AGTCAGA
7mer-2169
GTCAGA
6mer-543
CAGGTG
6mer-2978
HP26
Target
5'-CTATCCGATCCATCAAGTTCAGTCTCGAAAGAGTCTCAACATTTGTCCACAGCGTA-3'
AGTCAGA
7mer-2169
GTCAGA
6mer-543
CAGGTG
6mer-2978
HP28
Target
5'-CTATCCGATCCATCAAGTTCAGTCTCGAAACAGTCTCAACATTTGTCCACAGCGTA-3'
AGTCAGA
7mer-2169
6mer-2978
CAGGTC
GTCAGA
6mer-543
TarZT
Target
5'-TTGTCCACAGTTCAGTCTCAGAGCCTATCG -3'

Figure 12

6-mer 543



7-mer 2169



6-mer 2978

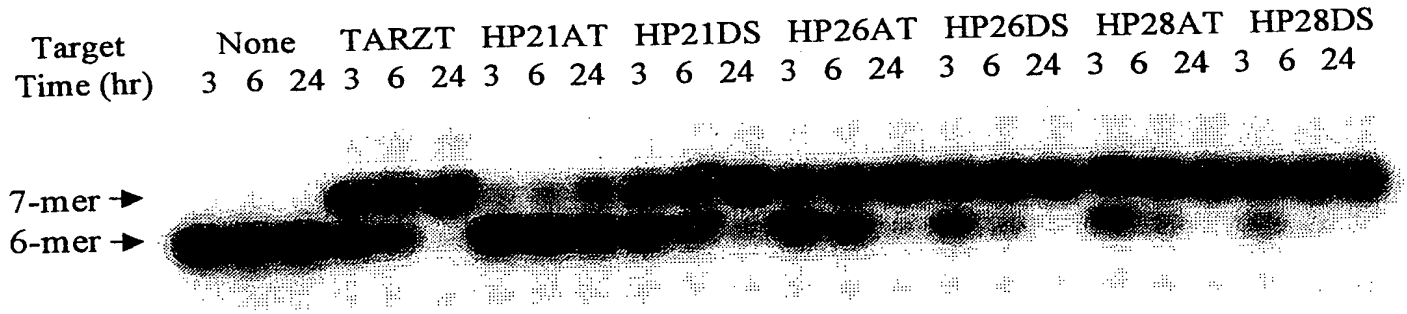
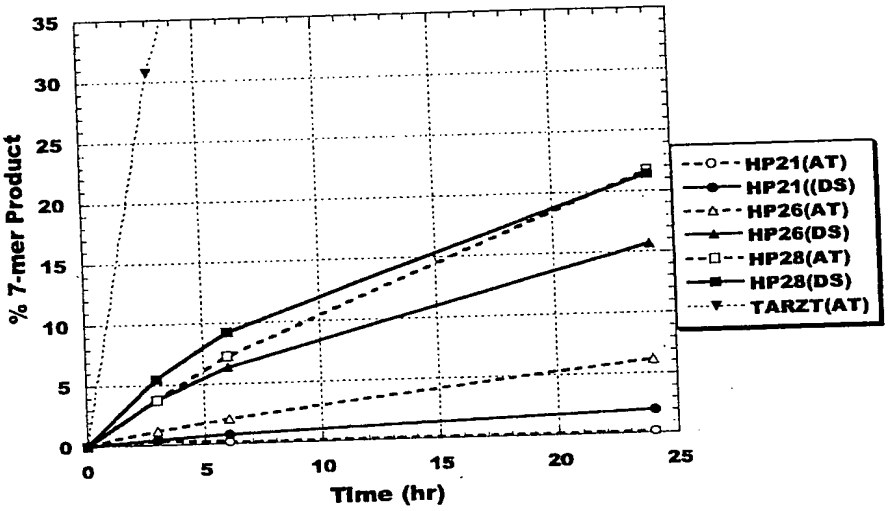
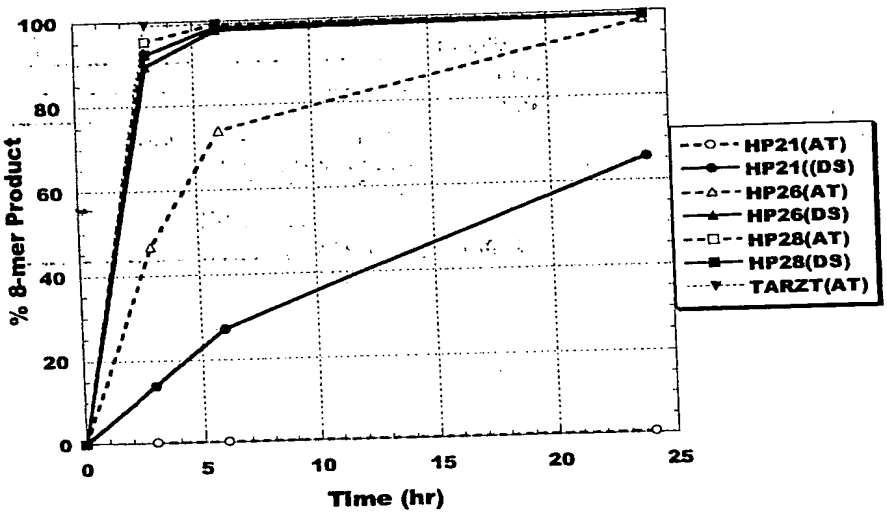


Figure 13

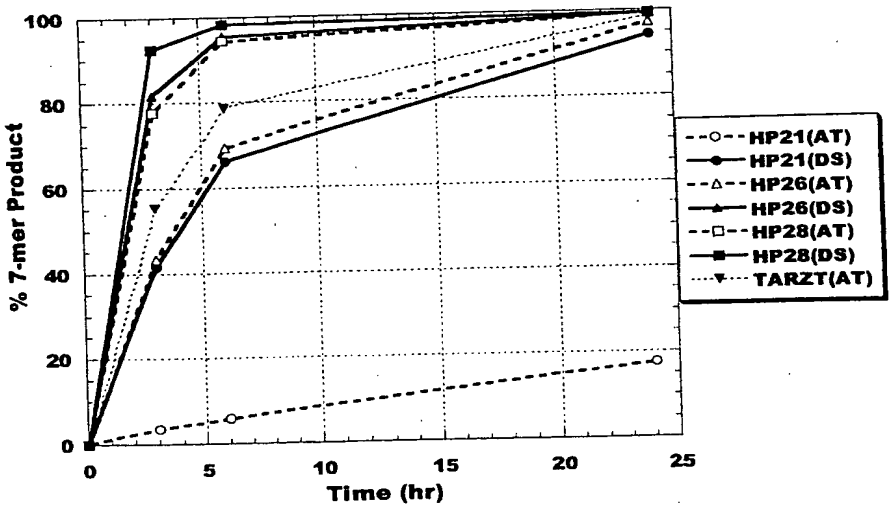
6mer-543



7mer-2169



6mer-2978



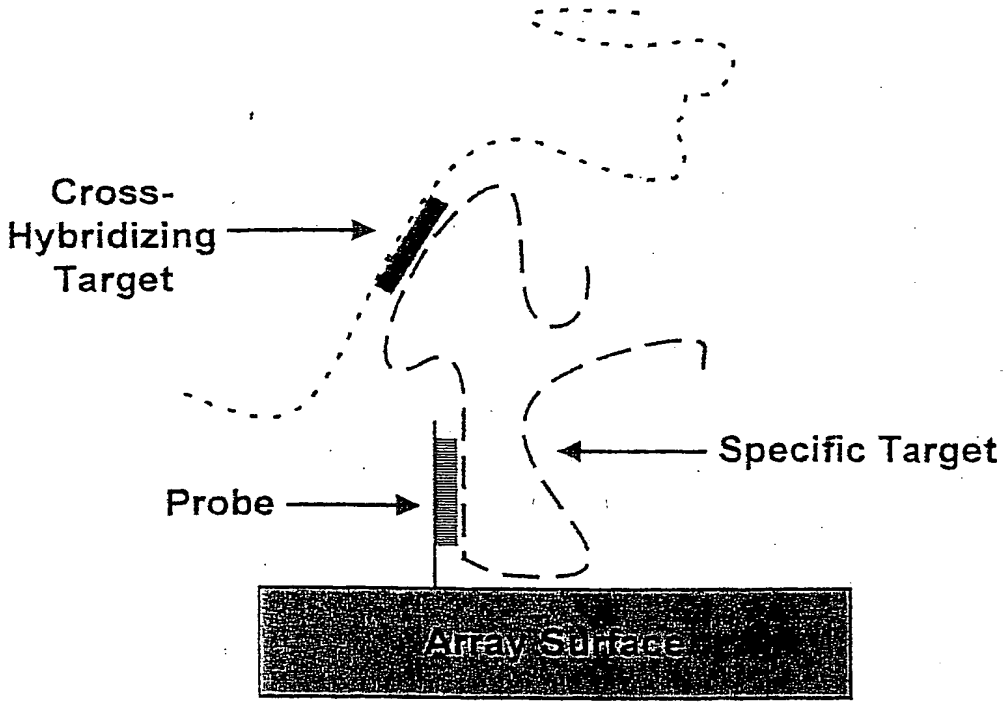

Cross-
Hybridizing
Target
Specific Target
Probe
Array Surface

Figure 14

Chemical & Biological Systems Department

DNA Hairpin Structure (HP51)

HP51 DNA

Predicted for 1M NaCl

G° = -12.3 kcal/mole at 37 °C
H° = -82.5 kcal/mole
S° = -226.3 cal/ (°K·mol)
Tm = 91.3°C

```
10         20
CCGATCCATCAA|          G
            GTTCAGTCTC A
             ||||||||||
            CAAGTCAGAG A
----------CA^          A
                    30
```

HP51 UNA

```
10         20
CCGATCCATCDD|          G
            GSSCDGSCSC D
             |  | | | |
            CDDGSCDGDG D
----------CD^          D
                    30 Z
```

Figure 15

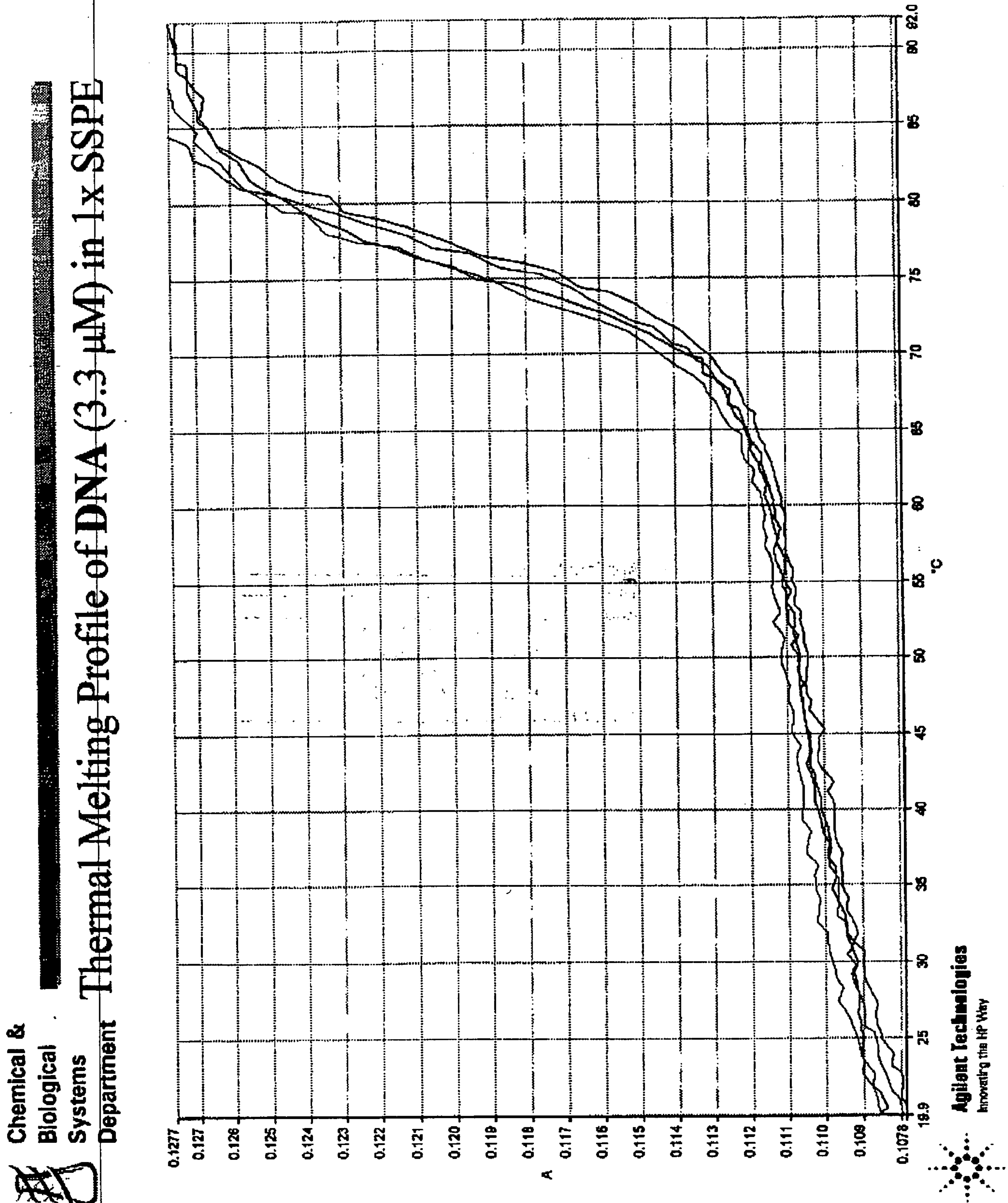

Chemical &
Biological
Systems
Department
Thermal Melting Profile of DNA (3.3 μM) in 1x SSPE
A
0.1277
0.127
0.126
0.125
0.124
0.123
0.122
0.121
0.120
0.119
0.118
0.117
0.116
0.115
0.114
0.113
0.112
0.111
0.110
0.109
0.1078
°C
19.9
25
30
35
40
45
50
55
60
65
70
75
80
85
90
92.0
Agilent Technologies
Innovating the HP Way

Figure 16

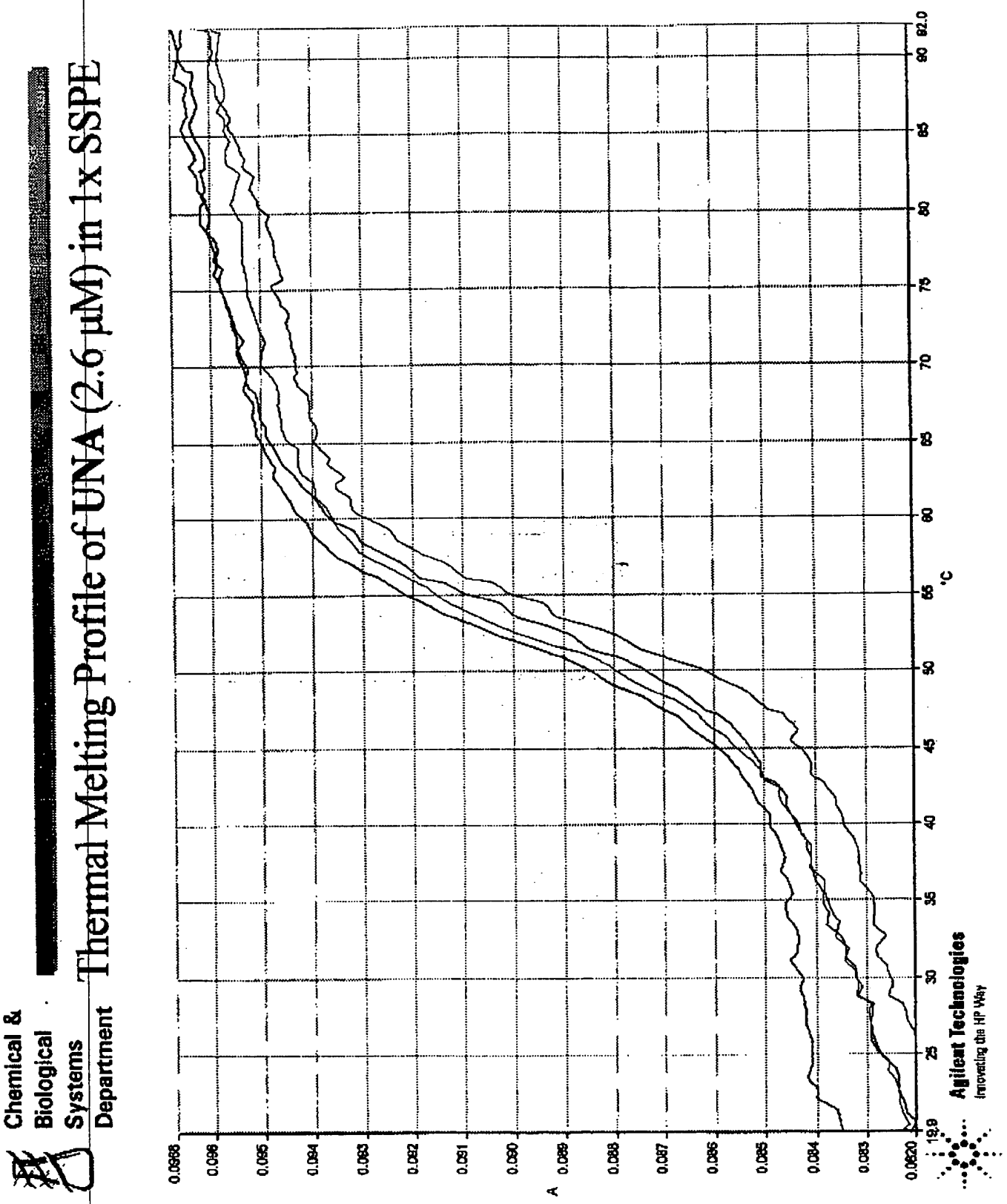


Figure 17

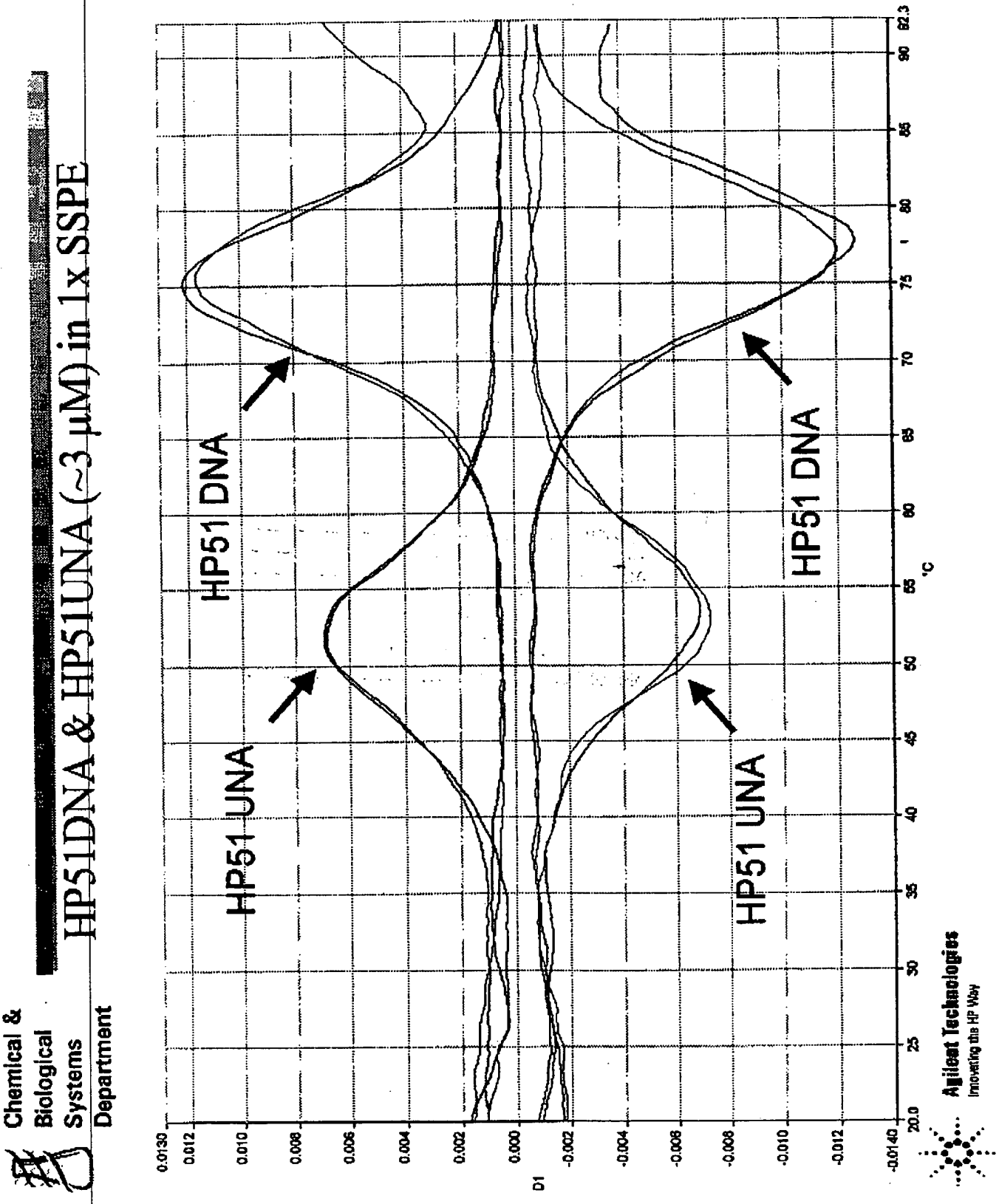

HP51DNA & HP51UNA (~3 µM) in 1x SSPE
Chemical & Biological Systems Department
HP51 DNA
HP51 UNA
HP51 UNA
HP51 DNA
D1
°C
Agilent Technologies
Inventing the HP Way

figure 18a

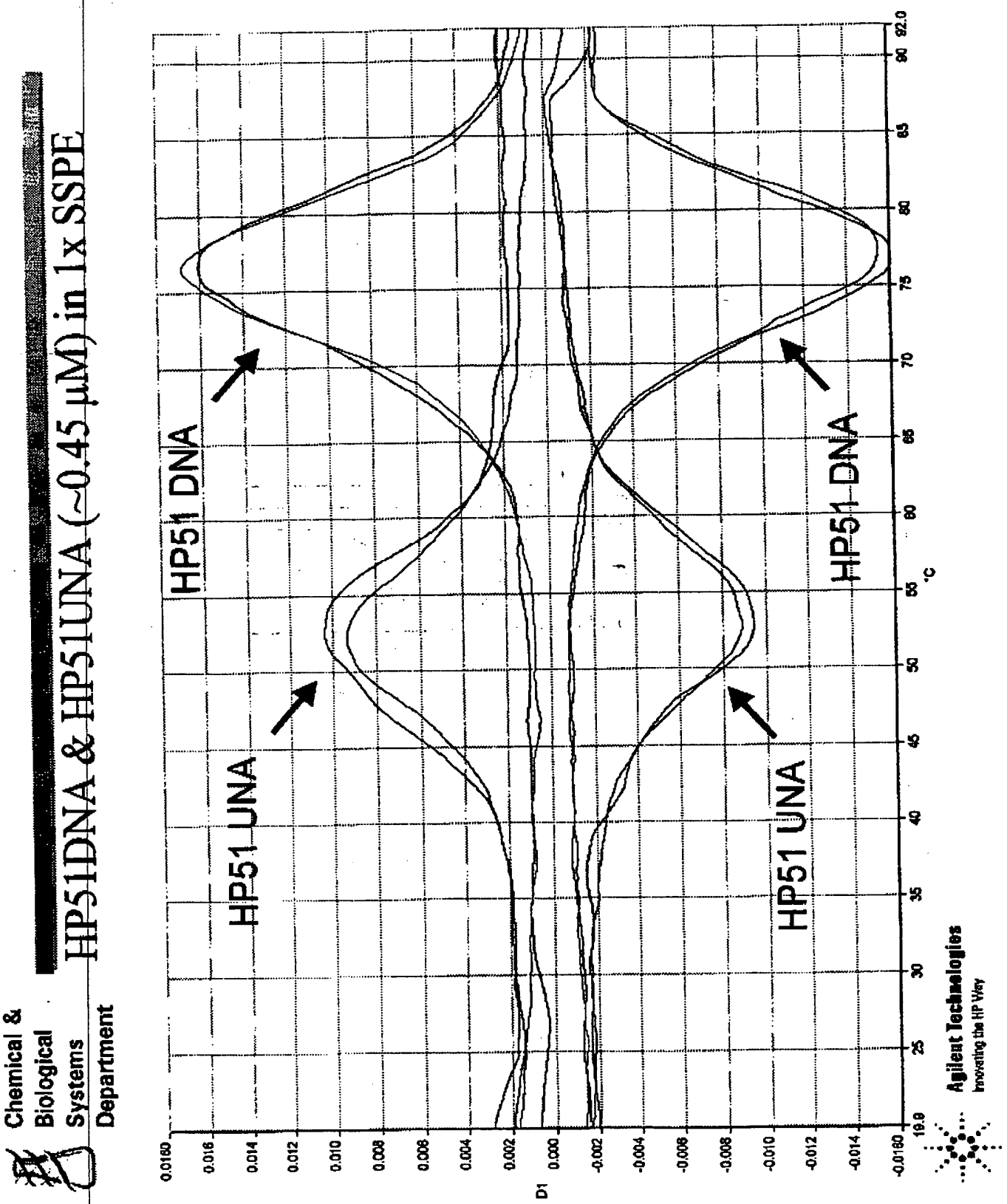

HP51DNA & HP51UNA (~0.45 µM) in 1x SSPE
Chemical & Biological Systems Department
HP51 DNA
HP51 UNA
HP51 UNA
HP51 DNA
D1
°C
Agilent Technologies
Innovating the HP Way

Figure 186

Sample	Ramp Up 1 Tm	Ramp Up 2 Tm	Ramp Dwn 1 Tm	Ramp Dwn 2 Tm	Average Tm
HP51 DNA	75.0 °C	75.5 °C	77.0 °C	78.0 °C	**76.4 °C**
HP51 UNA	52.0 °C	52.0 °C	53.0 °C	54.0 °C	**52.8 °C**
ΔTm	23.0 °C	23.5 °C	24.0 °C	24.0 °C	**23.6 °C**

figure 19